

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Microbiological features associated with the suppression of Fusarium wilt of lettuce studied by applying a single selected compost.

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1578705> since 2020-04-03T19:11:29Z

Published version:

DOI:10.1080/1065657X.2015.1122556.

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Microbiological features of compost-mediated suppression to *Fusarium* wilt of lettuce**

2

3 Selma Franceschini¹ selma.franceschini@unito.it

4 Massimo Pugliese^{1,2} massimo.pugliese@unito.it

5 Angelo Garibaldi¹ angelo.garibaldi@unito.it

6 Maria Lodovica Gullino^{1,2} marialodovica.gullino@unito.it

7

8 ¹AGROINNOVA

9 Centre of Competence for Innovation in the Agro-Environmental Sector

10 Università degli Studi di Torino

11 Via Leonardo da Vinci,44

12 10095 Grugliasco (TO) - Italia

13 Office: +39-0116708544

14 Fax +39 0116709307

15

16 ²DISAFA

17 Department of Agricultural, Forestry and Food Sciences

18 Università degli Studi di Torino

19 Via Leonardo da Vinci,44

20 10095 Grugliasco (TO) - Italia

21 Office: +39-0116708544

22 Fax +39-0116709307

23

24

25

26

Abstract

27 The effect of compost suppressiveness toward the *Fusarium* wilt of lettuce was studied using a
28 polyphasic approach. Bioassays were carried out in controlled conditions to allow a standardization
29 of the environment in order to minimize disturbances and variability of soil microbial communities.
30 Compost addition demonstrated significant efficacy in controlling the disease. Microbial activities,
31 bacterial and fungal loads were quantified and correlated in a Principal Component Analysis in
32 order to clarify the correlation between original variables. The samples were well distinguished
33 between the substrates where the plants were grown and the rhizosphere samples.
34 DGGE was used to track microbial communities along the bioassays. The approach demonstrated to
35 be useful to detect either bacterial or fungal species that preferentially associated with roots of
36 seedlings, and hence involved in the compost-mediated suppression, with a high probability to be
37 vital microorganisms because not detected in the substrate where the plants were grown at the start
38 of the experiment.

39

40 *Introduction*

41 *Fusarium oxysporum* f. sp. *lactucae* is an economical important pathogen of *Lactuca sativa*. The
42 pathogen was reported for the first time in Japan in 1955, then in California in 1990. Elsewhere it is
43 present in Iran and Taiwan. It was first reported in Europe by Garibaldi (*et al.* 2002) but is was
44 found also in Latin America (Ventura and Costa 2008). The pathogen can be seedborne allowing
45 therefore an easy movement between continents (Garibaldi *et al.* 2004a).

46 Disease control is normally carried out through crop rotation and genetic resistance (Scott *et al.*
47 2011). Among preventive measure, seed dressing have been proved to be successful in pathogen
48 control (Gilardi *et al.* 2005). The use of biological control agents is still in the experimental stage
49 (Fera 2011).

50 Compost, improving directly and indirectly soil properties, represents one of the most important
51 and cost-effective tool to control soil-borne plant pathogens and it is indeed one of the alternative to
52 be assayed in the control of *Fusarium* wilt of lettuce. In literature there are many examples of

53 containment of soilborne diseases resulting from the application of compost (Noble and Coventry
54 2005; Hadar and Papadopoulou 2012). Compost amendment acting either on the size and the
55 composition of soil microbial communities (Saison *et al.* 2006) can lead to control of plant diseases.
56 Compost suppressiveness varies depending on different pathosystems and compost applied
57 (Termorshuizen *et al.* 2006; Yogeve *et al.* 2006) but it is almost agreed in the scientific community
58 that the phenomenon of suppressiveness fully involves microbial communities. Among compost
59 bacteria the groups that majorly influence the development and suppressive activity of soil
60 microbial communities are *Bacillus* and *Pseudomonas* (Zaccardelli *et al.* 2013), but soil and
61 rhizosphere microbial associations could underlie to different shifts either in composition or size in
62 suppressive soils or substrates (Hagn *et al.* 2008; Meng *et al.* 2012; Klein *et al.* 2012).

63 Due to the complexity of the ecological framework in which soil microorganisms and plants
64 interact, polyphasic approaches should be adopted to study the mechanisms that drive the
65 suppressiveness of the disease (Garbeva *et al.* 2004; Boutler-Bitzer *et al.* 2006). The importance to
66 look in the rhizosphere to detect significant microbial associations and novel microorganisms
67 involved in disease control has been also pointed out recently (Huang *et al.* 2013; Mendes *et al.*
68 2013).

69 Polyphasic approaches applied to study soil and compost microbial population structures and
70 assemblages usually combine different biochemical, cultural and molecular assays with different
71 aims and resolutions (Saison *et al.* 2006; Nocker *et al.* 2007; Gardner *et al.* 2011; Gao *et al.* 2012
72 Suárez-Estrella *et al.* 2012).

73 Among biochemical assays fluorescein diacetate (FDA) can be hydrolysed by many enzymes
74 (lipases, proteases and esterases) and organisms providing a broad-spectrum indicator of soil and
75 compost biological activity (Schnürer and Rosswall 1982; Adam and Duncan 2001; Ryckeboer *et*
76 *al.* 2003; Komilis *et al.* 2011) but β -glucosidase is also considered a good enzymatic indicator
77 (Abellan *et al.* 2011; Gardner *et al.* 2011).

78 Among community-level molecular techniques applied in microbial ecology, DGGE is, together
79 with T-RFLP one of the most widely used PCR-based method for the study of fungal and bacterial
80 assemblages (Marshall *et al.* 2003; Cherif *et al.* 2008; Danon *et al.* 2010; Maeda *et al.* 2010;
81 Ferrocino *et al.* 2013).

82 The aim of the present study was: 1) to assay the applicability of municipal waste-based compost
83 against *Fusarium oxysporum* f. ap. *lactucae*; 2) to typify the microbiological features that
84 characterize suppressive assays.

85 To reach those objectives experimental trials, in presence and absence of compost, have been
86 conducted in growth chamber and microbiological parameters have been monitored along the
87 experiment in order to detect differences that could be attributed to the observed suppressive effect
88 of compost. DGGE profiles have been obtained for either the substrates at the beginning of the
89 experiments or for the rhizosphere samples in order to detect the active microflora involved in the
90 process.

91 The combined approach adopted in this study demonstrates to be effective to typify suppressive
92 substrate on the base of microbiological features while DGGE suggests the involvement of either
93 fungi, especially *Simplicillium lamellicola*, or bacteria, especially *Pseudomonas*, in the observed
94 control of the disease.

96 **Materials and methods**

97 *Experimental approach*

98 Compost used in this study was prepared from green wastes, organic domestic wastes and urban
99 sludges by ACEA Pinerolese SpA (Pinerolo, Torino, Italy). This compost was chosen because this
100 product showed a good suppressive activity in previous trials (Pugliese et al. 2007).

101

102 To assess the effect of compost amendment on disease development of *Fusarium* wilt of lettuce and
103 on the rhizosphere microbial communities, the experimental design included the presence and the
104 absence of the pathogen and the application of ACM compost at 0 and 1% concentration (v/v).
105 Experiments have been carried out in a climatic chamber at optimum temperature for pathogen
106 development ($27\pm 2^{\circ}\text{C}$). Disease progress was checked weekly.
107 Sampling were carried out at the time of plants transplant (Ti) and at the end of the disease
108 suppression experiment after 35 days (Tf).
109 At each sampling time following parameters were evaluated: a) enzymatic activities through the
110 Fluorescein Diacetate (FDA) hydrolysis and the β -glucosidase assays; b) estimate of the fungal and
111 bacteria total load; c) molecular fingerprinting of the bacterial and fungal population through the
112 application of DGGE technique.

113 114 *Growing media and inoculum preparation*

115 The isolate of *Fusarium oxysporum* f.sp. *lactucae* MYA30/40 was used in bioassays. The fungus
116 was grown in Potato Dextrose Broth. After two weeks the mycelia was collected through
117 centrifugation at 15000g for twenty minutes. A chlamydospore powder for inoculation was
118 produced adding talc in the ratio 4:1 respect to the fresh weight of the mycelia. The powder was
119 allowed to dry for further two weeks at room temperature and mixed thoroughly to obtain a fine and
120 homogenous inoculum source.
121 The concentration of the powder was assessed through dilution plating in Potato Dextrose Agar
122 amended with 60mg/L of streptomycin. The inoculum was added to the substrates to reach the final
123 concentration of 5×10^4 . Growing media consisted in steamed sandy soil to which the compost was
124 added at a rate of 100ml per liter. Control thesis did not contain the pathogen.
125 One month old lettuce seedlings of “crispilla bianca” variety were root washed to remove any peat
126 debris from the root and transplanted in 1 lt pots in number of 10 per thesis.

127 *In vivo* experiments were conducted twice in a climatic chamber at an optimum temperature for
128 growth of the pathogen and humidity-controlled (27 ° C, 80-90 % HR).

129

130 *Disease progress*

131 Plant disease assessment was carried out weekly following a disease scale of 5 points from 0
132 (healthy plant) to 4 (dead plant). The Area Under Disease progress Curve (AUDPC) and the
133 Suppressive Capacity (SC%) Termorshuizen *et al.* (2006).

134

135 *Sampling and microbiological parameters estimate*

136 Samples analyzed were collected at the time zero (Ti=Tinitial) of the experiment and at the end of
137 the experiment (Tf=Tfinal) either for inoculated or not inoculated theses. Ti samples were
138 representative of the mixtures used for setting the experiment whether at the Tf samples were
139 collected from the rhizosphere of plants (amended and not amended; inoculated and not) through
140 shaking the root apparatus in sterile petri dishes after carefully digging out the plant from the pot.
141 Single samples were composed of a bulk of the rhizosphere sample from the same thesis. The single
142 unit for all the microbiological and molecular assays was 0.5g.

143 Enzymatic activity was measured through FDA following the protocols of Ryckeboer *et al.* (2003)
144 and Adam *et al.* (2001) and β -glucosidase was carried out according to Andres Abellan *et al.*
145 (2011).

146 The total fungal and bacterial load were determined using the dilution plating technique. Briefly
147 0.5g of sample was suspended in 50ml of ¼ strength Ringer (Merk[®], Germany) solution and two
148 drops of tween (Sigma Aldrich[®], Germany) and incubated in a rotary shaker for one hour. Serial
149 dilution were spread on Potato Dextrose Agar amended with 60mg/L of streptomycin for fungi and
150 Luria Bertani Agar for bacteria.

151

152 *DGGE*

153 DNA was extracted from each sample unit using NUCLEO SPIN SOIL KIT (Macherey-Nagel GmbH &
154 Co. KG) following manufacturers' instructions, with a final elution step in MilliQ autoclaved water
155 as for fungal DNA.

156 Fungal DNA was amplified using NS1/GCFung primers according to the protocol of Gao *et al.*
157 (2012) while for bacteria 1070F/1392R as developed by Nocker *et al.* (2007) was used.

158 Each PCR mixture contained 10 ng of DNA, 2µl of primer 10 mM, 4µl 2.5mM DNTPS, 2.5µl of
159 MgCl₂, 5 µl of 10XBuffer, and water up to 50µl of reaction volume.

160 The amplicons (20µl), were loaded in 8% acrilammide, 30-70% gradient, gels for bacteria and in
161 6% acrilammide, 25-45% gradient, gels for fungi. In both cases the gels were run at 60°C for 16 h
162 at 75V. Images were acquired with Geldoc BIORAD®. DGGE bands were excised, re-amplified
163 and sequenced using the forward primer either for bacteria (1070F) or for fungi (NS1) to determine
164 the identity of dominant taxa.

165

166 *Statistical Analysis*

167 All datasets were assessed for the normality of variance (Shapiro Wilk W test) assuming 0.05 as α -
168 value. The data of the two experiments were pooled together after the verification of the
169 homogeneity of variances according to the Levene's test ($P>0.05$). Unless otherwise stated,
170 ANOVA and Tukey's post hoc test was used to verify the statistical significance of differences at a
171 $P<0.05$, unless otherwise specified. All statistical analyses were carried out with SPSS (IBM SPSS
172 Statistics 21, NY, US) except PCA that was elaborated with PAST (Hammer *et al.* 2001)

173

174 *Results and Discussion*

175 *Compost suppressive capacity*

176 Mean disease severity in the plants inoculated with *Fusarium oxysporum* f. sp. *lactucae* was $2.85 \pm$
177 0.37 for the not amended thesis and 1.55 ± 0.45 for the compost amended thesis. Level of disease
178 in untreated plants agreed with the susceptible behavior typical of the cultivar crispilla (Garibaldi *et*

179 *al.*, 2004b). The addition of 1% compost lead to a suppressive capacity of 41% with significant
180 differences between either the mean disease severity and the AUDPC values (Figure 1). No disease
181 were observed in control theses.

182

183 *Microbiological parameters*

184 All microbiological parameters were higher at the Tf (Table 1 and 2), confirming a highest
185 microbial activity around the roots of plants. As expected in general enzymatic activities
186 significantly higher in the 1% theses confirming the ability of the assay in detecting microbial
187 activity changes among samples. Specifically FDA and total fungal load were highest in the
188 rhizosphere of inoculated plants while beta-glucosidase and bacterial load was highest in the healthy
189 plants.

190 PCA analysis (Figure 2) allowed to resolve three main groups: a groups embodied by the samples
191 of substrates taken at the beginning of the experiment (1-4) and another group of samples embodied
192 by the rhizosphere samples (5-8). Among this last group samples were further subdivided in not
193 inoculated (6,7) and inoculated (7,8).

194 While the higher enzymatic activity (FDA) around the root is likely to be consequence of the
195 activation of microbial population that followed plant inoculation (Caravaca *et al.* 2006), the
196 highest concentration of beta-glucosidase enzyme in healthy plants might be probably the
197 consequence of the enhanced carbon and nutrient allocation into the rhizosphere due to better
198 growth conditions (Tscherko *et al.* 2003).

199

200 *DGGE profiling*

201 Bacterial population was composed mainly by *Flavobacteria* (Figure 3) either at the start or at the
202 end of the experiment. This is consistent with the recent findings of Gardner *et al.* (2011) who,
203 investigating the microbial communities in five different farming systems, discovered that
204 *Flavobacteria* are among the predominant bacterial phyla in the first 0-10 cm of soil, including

205 lettuce. DGGE profile of bacterial population suggest that *Pseudomonas* sp., alone or in association
 206 with *Flavobacterium* sp., could be involved in the process of suppression. In fact *Pseudomonas* was
 207 found only in the rhizosphere of 1% only while *Flavobacterium* spp. were present in all thesis (see
 208 arrows in figure 3). This result is in agreement with the proved affectivity of *Pseudomonas* in
 209 controlling wilt pathogens (Srinivasan *et al.* 2009; Karimi *et al.* 2012; Mansoori *et al.* 2013).
 210 ITS primers chosen in this study amplified DNA from deuteromycetes (*Verticillium*, *Fusarium*,
 211 *Simplicillium*) but also other organisms such as the yeast *Metschnikowia chrysoperlae*, the lichen
 212 *Dyctionema sericeum*, other the ascomycete *Peziza basisiofusca*.
 213 The highest number of fungal taxa was recorded for lettuce plants inoculated and grown in soil
 214 amended with 1% of compost. According to DGGE profiling *Verticillium* (Figure 4) is fungal genus
 215 that preferentially associates to the roots of lettuce plants.
 216 Among other fungal taxa *Simplicillium lamellicola* was the only species detected in the rhizosphere
 217 of plants challenged with the pathogen and treated with 1% compost, suggesting that it also may
 218 specifically contribute to the process of disease suppression.
 219 As expected not all DGGE bands resulted in known organisms. In details one band in the bacterial
 220 (2) and two in the fungal fingerprints (2 and 3) resulted in uncultured bacterium, uncultured fungi to
 221 an ascomycete respectively. This is possible because also unknown and uncultured organisms could
 222 be detected with the use of molecular techniques.

223

224 *Summary*

225 ACM compost added to the percentage of 1% to steamed sandy soil allowed to obtain around 40%
 226 disease control of *Fusarium oxysporum* f.sp. *lactucae* “in vivo” demonstrating that ACM compost
 227 can be considered as alternative or integrative of resistant cultivars for controlling the disease. PCA
 228 analysis of microbiological features showed that all samples taken from the rhizosphere of plants at
 229 the end of the experiment were more active than the respective samples taken as representative of
 230 the substrates only. Samples obtained from the rhizosphere of plants grown in suppressive media

231 were characterized by highest total enzymatic activity and highest load of total fungi. The
232 comparison of DGGE profiles of microbial populations of the thesis has revealed a greater diversity
233 to the fungal community than that of bacteria. *Pseudomonas* sp., among bacteria, and *Simplicillum*
234 *lamellicola*, among deuteromycetes, were detected only in the rhizosphere of plants treated with 1%
235 compost indicating that they may play an active role disease control. Further studies are needed in
236 order to clarify the involvement of these two species in the compost-mediated disease suppression
237 of *Fusarium* wilt of lettuce.

238

239 **Acknowledgments**

240 This work was conducted thanks to the funds of project

241

242

243 **References**

- 244 Abellan, M.A., C. W. Baena, F.A.C. Morote, M.I. P. Cordoba, D.C. Perez and M.E. Lucas-Borjia.
245 2011. Influence of soil storage method on soil enzymatic activities in mediterranean forest
246 soils. *Forest Systems*, 20(3): 379–388.
- 247 Adam, G. and H. Duncan. 2001. Development of a sensitive and rapid method for the measurement
248 of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology*
249 *and Biochemistry*. 33:943–951.
- 250 Boutler-Bitzer, J.I., J.T. Trevors and G.J. Boland. 2006. A polyphasic approach for assessing
251 maturity and stability in compost intended for suppression of plant pathogens. *Appl. Soil*
252 *Ecol.* 34: 65–81.
- 253 Caravaca, F., M.M. Alguacil, R. Azco and A. Rolda. 2006. Formation of stable aggregates in
254 rhizosphere soil of Junipers oxycedrus: Effect of AM fungi and organic amendments. *Appl.*
255 *Soil Ecol.*, 33:30–38.

256 Cherif, H., H. Ouzari, M. Mazorati, L. Brusetti, N. Jedidi, A. Hassen and D. Daffonchio. 2008.
 257 Bacterial community diversity assesement in municipal solid waste compost amended soil
 258 using DGGE and ARISA. *World J. Microbiol. Biotechnol.*, 24: 1159–1167.

259 Fera. 2011. Rapid assessment of the need for a detailed Pest Risk Analysis for *Fusarium oxysporum*
 260 f.sp. *lactucaae*. Registered file PPP 13857.

261 Ferrocino, I., W. Chitarra, M. Pugliese, G. Gilardi, M. L. Gullino and A. Garibaldi. 2013. Effect of
 262 elevated atmospheric CO₂ and temperature on disease severity of *Fusarium oxysporum* f.sp.
 263 *lactucaae* on lettuce plants. *Appl. Soil Ecol.*, 72: 1–6

264 Gao, G., D. Yin, S. Chen, F. Xia, Q. Li, and W. Wang. 2012. Effect of biocontrol agent
 265 *Pseudomonas fluorescens* 2P24 on soil fungal community in cucumber rhizosphere using T-
 266 RFLP and DGGE. *PLOS One* 7(2):e31806.

267 Garbeva, P., J.A. Van Veen and J.D. Van Elsas. 2004. Microbial diversity in soil: selection of
 268 microbial populations by plant and soil type and implications for disease suppressiveness.
 269 *Annu. Rev. Phytopathol.*, 42: 243–270.

270 Gardner, T., V. Acosta-Martinez, Z. Senwo and S.E. Dowd. 2011. Soil Rhizosphere microbial
 271 communities and enzyme activities. *Diversity*, 3:308–328.

272 Garibaldi, A., G. Gilardi and M.L. Gullino. 2004a. Seed transmission of *Fusarium oxysporum* f. sp.
 273 *lactucaae*. *Phytoparasitica* 32: 61–5.

274 Garibaldi, A., G. Gilardi and M.L. Gullino. 2004b. Varietal resistance of lettuce to *Fusarium*
 275 *oxysporum* f. sp. *lactucaae*. *Crop Prot.* 23: 845–51.

276 Garibaldi, A., G. Gilardi, and M.L. Gullino. 2002. First report of *Fusarium oxysporum* on lettuce in
 277 Europe. *Plant Dis.* 86, 1052.

278 Gilardi, G., F. Tinivella, M. L. Gullino and A. Garibaldi. 2009. Seed dressing to control *Fusarium*
 279 *oxysporum* f. sp. *lactucaae*. *J. Plant Dis.Prot.*, 112(3): 240–246.

280 Hadar, Y. and K.K. Papadopoulou. 2012. Suppressive composts: microbial ecology links between
 281 abiotic environments and healthy plants. *Annu. Rev. Phytopathol.*, 50:133–153.

282 Hagn, A., M. Engel, B. Kleikamp, J.C. Munch, M. Scholoter and C. Bruns. 2008. Microbial
 283 community shifts in *Pythium ultimum*-inoculated suppressive substrates. *Biol. Fertil. Soils*,
 284 44: 481–490.

285 Hammer, O., D.A.T. Harper and P.D. Ryan. 2001. PAST: Paleontological Statistic software
 286 package for education and data analysis. *Paleontologia Eletronica* 4(1): 1-9. [http://palaeo-](http://palaeo-electronica.org/2001_1/past/issue1_01.htm)
 287 [electronica.org/2001_1/past/issue1_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm) [Accessed: 04/X/2011]

288 Huang, J., Z. Wei, S. Tan, X. Mei, S. Yin, Q. Shen, and Y. Xu. 2013. The rhizosphere soil of
 289 diseased tomato plants as source for novel microroganisms to control bacterial wilt. *Appl.*
 290 *Soil Ecol.*, 72: 79–84.

291 Karimi, K., J. Amini, B. Harigi and B. Bahramnejad. 2012. Evaluation of biocontrol potential of
 292 *Pseudomonas* and *Bacillua* spp. against *Fusarium* wilt of chickpea. *Austr. J. Crop Sci.*, 6(4):
 293 695–703.

294 Klein, E., M. Ofek, J. Katan, D. Minz and A. Gamliel. 2013. Soil suppressiveness to *Fusarium*
 295 Disease: shifts in root microbiome associated with the reduction of pathogen root
 296 colonization. *Biol. Control*, 103(1):23–33.

297 Komilis, D., I. Kontou and S. Ntougias. 2011. A modified static respiration assay and its
 298 relationship with an enzymatic test to assess compost stability and maturity. *Bioresource*
 299 *Technol.*, 102:5863–5872.

300 Maeda, K., D. Hanajima, R. Morioka and T. Osada. 2010. Characterisation and spatial distribution
 301 of bacterial communities within passively aereted cattle manure composting piles.
 302 *Bioresource Technol.*, 101: 9631–9637.

303 Mansoori, M., A. Heydari, N. Hassanzadeh, S. Rezaee and L. Naraghi. 2013. Evaluation of
 304 *Pseudomonas* and *Bacillus* bacterial antagonists for biological control of cotton *Verticillium*
 305 wilt disease. *J. Plant Prot. Res.*, 53(2)154–157.

306 Marshall, M.N., L. Cocolin, D.A. Mills and J.S. Vandergheynst. 2003. Evaluation of PCR primers
 307 for denaturing gradient gel electrophoresis analysis of fungal communities in compost. *J.*
 308 *Appl. Microbiol.*, 95: 934–948.

309 Mendes, R., P. Garbeva and J.M. Raaijmakers. 2013. The rhizosphere microbiome: significance of
 310 plant beneficial and plant pathogenic, and human pathogenic microorganisms. *FEMS*
 311 *Microbiol. Rev.*, 37: 634–663.

312 Meng, Q.X., J.F. Yin, N. Rosenzweig, D. Douches and J.J. Hao. 2012. Culture-based assessment of
 313 microbial communities in soil suppressive to potato common scab. *Plant Dis.*, 96(5): 712–
 314 717.

315 Noble, R. and E. Coventry. 2005. Suppression of soil-borne plant diseases with composts: a review.
 316 *Biocontrol Sci. Technol.*, 15(1): 3–20.

317 Nocker, A., P. Sossa-Fernandez, M.D. Burr and A.K. Camper. 2007. Use of propidium monoazide
 318 for live/dead distinction in Microbial ecology. *Appl. Environ. Microbiol.*, 5111–5117.

319 Pugliese, M., A. Garibaldi and M.L. Gullino, 2007. The use of com post in horticulture for
 320 controlling soil-borne pathogens. *Phytopathol.*, 97:s95.

321 Ryckeboer, J., J. Mergaert, J. Coosemans, K. Deprins and J. Swings. 2003. Microbiological aspects
 322 of biowaste during composting in a monitored compost bin. *J. Appl. Microbiol.*, 94: 127–
 323 137.

324 Saison, C., V. Degrange, R. Oliver, P. Millard, C. Commeaux, D. Montange and X. Le Roux. 2006.
 325 Alteration and resilience of the soil microbial community following compost amendment:
 326 effects of compost levele ad compost-borne microbial community. *Environ. Microbiol.*,
 327 8(2): 247–257.

328 Schnürer, J., and T. Rosswall. Fluorescein diacetate hydrolysis as a measure of Total microbial
 329 activity in soil and litter. *Appl. Environ. Microb.*, 43(6): 1256–1261.

330 Scott, J.C., S.C. Kirkpatrick and T.R. Gordon. 2010. Variation in susceptibility of lettuce cultivars to
 331 fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae*. *Plant Pathol.*, 59: 139–146.

332 Srinivasan, K., G. Gilardi, A. Garibaldi, and M.L. Gullino. 2009. Bacterial antagonists from used
333 rockwool soilless substrates suppress *Fusarium* wilt of tomato. *J. Plant Pathol.*, 91(1): 147–
334 154.

335 Suárez-Estrella, F., M.A. Bustamante, R. Moral, M.C. Vargas-García, M.J. López and J. Moreno.
336 2012. In vitro control of fusarium wilt using agroindustrial subproduct-based composts. *J.*
337 *Plant Pathol.*, 94(1): 59–70.

338 Tscherko, D., U. Hammesfahr, M.C. Marx and E. Kandeler. 2003. Shifts in rhizosphere microbial
339 communities and enzyme activity of *Poa alpina* across alpine chronosequence. In: 2nd
340 Enzymes in the Environment Conference, Prague, CZECH REPUBLIC, 1685–1698.

341 Ventura J, Costa H, 2008. Fusarium wilt caused by *Fusarium oxysporum* on lettuce in Espírito
342 Santo, Brazil. *Plant Dis.*, 92: 976.

343 Yogev, A., M. Raviv, Y. Hadar, R. Cohen and J. Katan. 2006. Plant waste-based composts
344 suppressive to diseases caused by pathogenic *Fusarium oxysporum*. *Eur. J. Plant Pathol.*,
345 116: 267–278.

346 Zaccardelli, M., F. De Nicola, D. Vilecco and R. Scotti. The development and suppressive activity
347 of soil microbial communities under compost amendment. *J. Soil Sci. Plant Nutr.*, 13(3):
348 730–742.

349

350

351

352

353

354

355

356

357

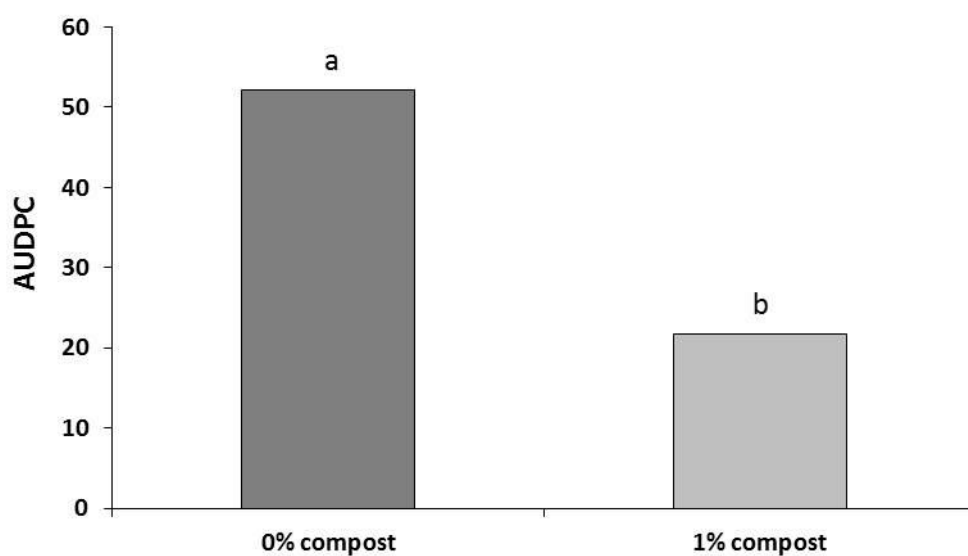
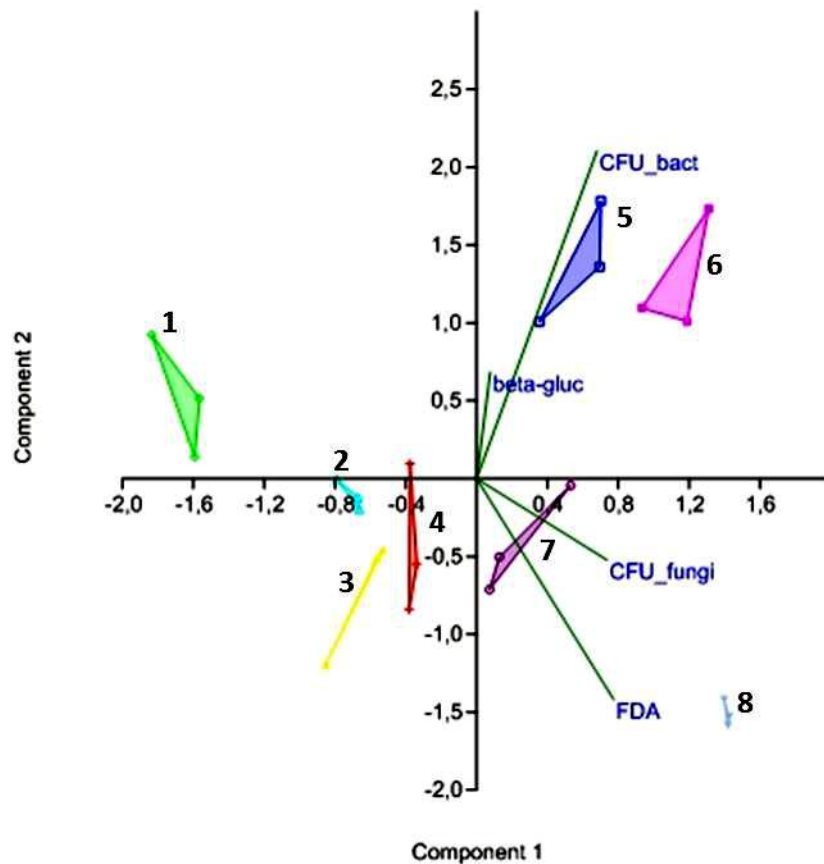


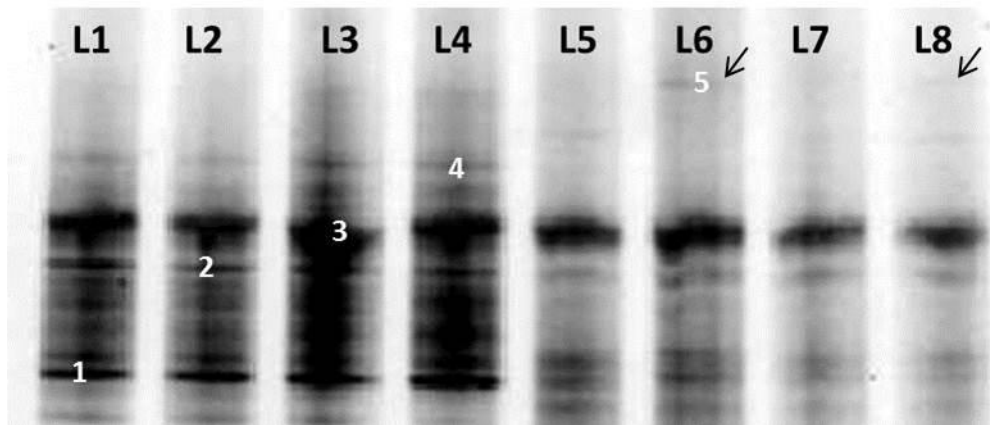
FIGURE 1. Mean AUDPC values for plants inoculated and grown in soil non amended (0%) and amended with 1% of ACM compost. Different letters above the column indicate significant ($P < 0.02$) differences between groups of values (Mann Whitney test).



367

368 FIGURE 2. Principal Component Analysis scattered plot of the microbiological parameters assessed
 369 for soil and rhizosphere samples during suppressive assays on lettuce plants challenged with
 370 *Fusarium oxysporum* f.sp. *lactucae*. Numbers correspond to treatments/samples: 1) Ti-not
 371 inoculated-0% compost-no plant; 2) Ti-not inoculated-1% compost-no plant; 3) Ti-inoculated-0%
 372 compost-no plant; 4) Ti-inoculated-1% compost-no plant; 5) Tf-not inoculated-0% compost-
 373 rhizosphere; 6) Tf-not inoculated-1% compost-rhizosphere; 7) Tf-inoculated-0% compost-rhizosphere;
 374 8) Tf-inoculated 1%-compost-rhizosphere.

375



376

377 FIGURE 3. DGGE profiles of bacterial community at the Time zero (Ti) lines 1 to 4 and at the end
 378 of the experiment (Tf) lines 5 to 8. L1) Ti-not inoculated-0% compost-no plant; L2) Ti-not
 379 inoculated-1% compost-no plant; L3) Ti-inoculated-0% compost-no plant; L4) Ti-inoculated-1%
 380 compost-no plant; L5) Tf-not inoculated-0% compost-rhizosphere; L6) Tf-not inoculated-1%
 381 compost-rhizosphere; L7) Tf-inoculated-0% compost-rhizosphere; L8) Tf-inoculated 1%-compost-
 382 rhizosphere. 1) *Flavobacterium tiangeerense*; 2) Uncultured bacterium; 3) *Flavobacterium*
 383 *xinjangense*; 4) *Flavobacterium* sp. 5) *Pseudomonas* sp..

384

385

386

387

388

389

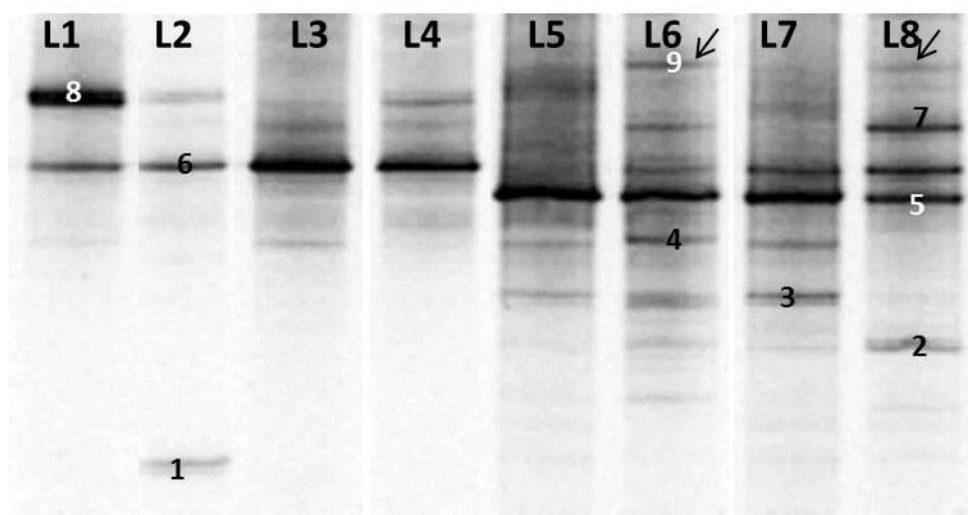
390

391

392

393

394



395

396 FIGURE 4. DGGE profiles of fungal community at the Time zero (Ti) lines 1 to 4 and at the end of
 397 the experiment (Tf) lines 5 to 8. L1) Ti-not inoculated-0% compost-no plant; L2) Ti-not inoculated-
 398 1% compost-no plant; L3) Ti-inoculated-0% compost-no plant; L4) Ti-inoculated-1% compost-no
 399 plant; L5) Tf-not inoculated-0% compost-rhizosphere; L6) Tf-not inoculated-1% compost-
 400 rhizosphere; L7) Tf-inoculated-0% compost-rhizosphere; L8) Tf-inoculated 1%-compost-rhizosphere.
 401 1) *Metschinkowia chrysoperlae*; 2) Uncultured fungus; 3) Ascomycete; 4) *Verticillium* sp.; 5)
 402 *Verticillium* sp; 6) *Fusarium oxysporum*; 7) *Peziza basidiofusca*; 8) *Dyctionema sericeum*; 9)
 403 *Simplicillium lamellicola*.

404

405

406

407

408

409

410

411

412

413

TABLE 1. Statistical differences in the mean enzymatic activities (\pm SD) as assessed through the FDA hydrolysis and betaglucosidase assays at the time zero (Ti) and at the end of the experiment (Tf). Different letters represent statistical differences at $P<0.05$ (ANOVA-Tukey's honestly post-hoc test).

FDA ($\mu\text{g/mlh}^{-1}$)					Beta-glucosidase ($\mu\text{g/mlh}^{-1}$)	
compost			Ti	Tf	Ti	Tf
(Ti) substrate	control	0%	7.30±0.02 e	-	0.22±0.01 ef	-
		1%	7.67±0.03 c	-	0.35±0.01 b	-
	inoc	0%	7.76± 0.05 c	-	0.27±0.04 d	-
		1%	7.72±0.03 c	-	0.28±0.02 cd	-
(Tf) plant rhizospere	control	0%	-	8.13±0.04 b	-	0.75±0.02 a
		1%	-	8.17±0.07 b	-	0.34±0.02 bc
	inoc	0%	-	8.09±0.03 b	-	0.20±0.01 f
		1%	-	9.22± 0.01 a	-	0.28±0.04 cd

TABLE 2. Statistical differences in the mean CFU of fungi and bacteria at the time zero (Ti) and at the end of the experiment (Tf). Different letters represent statistical differences at $P<0.05$ (ANOVA- Tukey's honestly post-hoc test).

			Total bacteria (CFU/gfw)		Total fungi (CFU/gfw)	
compost			Ti	Tf	Ti	Tf
(Ti) substrate	control	0%	6.58±0.11 de	-	2.64±0.30 d	-
		1%	6.69±0.01 de	-	3.43±0.08 d	-
	inoc	0%	6.72±0.20 de	-	3.78±0.25 bcd	-
		1%	6.50±0.20 e	-	3.65±0.09 cd	-
(Tf) plant rhizosphere	control	0%	-	7.66±0.20 ab	-	3.77±0.07 bcd
		1%	-	7.91±0.18 a	-	4.31±0.12 a
	inoc	0%	-	7.02±0.21 cd	-	4.00±0.13 abc
		1%	-	7.27±0.04 bc	-	4.16±0.05 ab